

Denitrification and the Dinitrogen/Nitrous Oxide Ratio as Affected by Soil Water, Available Carbon, and Nitrate

K. L. Weier,* J. W. Doran, J. F. Power, and D. T. Walters

ABSTRACT

Biological denitrification is affected by many environmental factors that control the amount of N_2 and N_2O entering the atmosphere. This study was conducted to measure the effect of water-filled pore space (WFPS), available C, and soil NO_3 concentration on total denitrification ($N_2 + N_2O$), using acetylene (C_2H_2) inhibition, and to ascertain if denitrification could be estimated from N_2O measurements in the field using an average N_2/N_2O ratio. Repacked cores of four benchmark soils were brought to 60, 75, and 90% WFPS by applying treatments of glucose-C (0, 180, and 360 kg ha⁻¹) and NO_3 -N (0, 50, and 100 kg ha⁻¹). The cores were incubated at 25 °C, with and without 100 mL C_2H_2 L⁻¹, for 5 d during which daily gas samples of the headspace were analyzed for N_2O and CO_2 . Total N loss due to denitrification generally increased as soil texture became finer and WFPS increased. The only exception to this was the C-amended sand, where N losses up to 26 and 66% were recorded at 60 and 75% WFPS, respectively. Denitrification rates at high N concentrations were quite small in the absence of an available C source but increased with increasing available C (glucose). The N_2/N_2O ratio generally increased with time of incubation after the initial treatment application. The largest ratios (up to 549) were found at the highest available C rate and generally at the highest soil water content. The presence of high NO_3 concentrations apparently inhibited the conversion of N_2O to N_2 , resulting in lower N_2/N_2O ratios. Using an average N_2/N_2O ratio for estimation of denitrification from N_2O field measurements cannot be recommended because of the variation in this ratio due to the many environmental factors altered by field management that influence denitrification and the relative production of N_2 and N_2O .

DENITRIFICATION is the major biological process by which N escapes from the soil and is the prin-

K.L. Weier, CSIRO, Div. of Tropical Crops and Pastures, 306 Carmody Rd., St. Lucia, Queensland, Australia 4067; J.W. Doran and J.F. Power, USDA-ARS, 119 Keim Hall, Univ. of Nebraska, Lincoln, NE 68583; and D.T. Walters, Univ. of Nebraska, Lincoln, NE 68583. Published as Paper No. 9865, Journal Series, Nebraska Agricultural Research Division, Lincoln. Received 10 Feb. 1992. *Corresponding author.

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cipal pathway by which the environmental pollutant N_2O enters the atmosphere (Bouwman, 1990). Eichner (1990) estimates that fertilizer-derived emissions of N_2O in the year 2000 will account for 0.1 to 1.5% of the global source and will probably not exceed 3% N_2O -N in the atmosphere. The proportion of denitrification gases entering the atmosphere as N_2O and N_2 , however, is dependent (on a number of environmental factors (Arah and Smith, 1990).

Early investigations on denitrification by Wijler and Delwiche (1954) and Nommik (1956) centered on the individual effects of pH, NO_3 concentration, the presence of an available energy source, and soil water and temperature. Since then, research has intensified and there is now a good understanding of the influence of these variables on denitrification (Delwiche and Bryan, 1976; Blackmer and Bremner, 1978; Burford and Bremner, 1975; Linn and Doran, 1984; Keeney et al., 1979). The effect of different concentrations of C (glucose) and NO_3 on the above process has also been studied (Bowman and Focht, 1974; Lalisse-Grundmann et al., 1988). In a field study, Weier et al. (1991) observed a direct relationship between N_2O production, monthly rainfall, and soil NO_3 concentration. However, there is limited information on the effect of combinations of more than two factors on denitrification.

The ratio of N_2/N_2O evolution from soils during the denitrification process is also affected by these environmental factors (Firestone et al., 1979; Letey et al., 1980; Vinther, 1984). Constancy of the N_2/N_2O ratio is important in estimating denitrification losses from field soils where only N_2O emissions into the atmosphere are measured (Ryden et al., 1979).

The objective of our research was to measure the effect of available C, soil NO_3 , and WFPS on total denitrification ($N_2 + N_2O$) in four soils using C_2H_2 inhibition, and to establish whether an average $N_2/$

Table 1. Characteristics of the benchmark soils used for this experiment.

Soil series	Classification	pH	Experimental bulk density Mg m ⁻³	Mineral N†		Textural analysis		
				NO ₃	NH ₄	Sand	Silt	Clay
				mg kg ⁻¹		%		
Valentine sand	Typic mixed, mesic Ustipsamment	7.4	1.35	2.5	1.4	90	7	3
Hord silt loam	Cumulic fine-silty, mixed, mesic Haplustoll	7.3	1.15	5.8	0.2	34	46	20
Yolo silt loam	Typic fine-silty, mixed, nonacid, thermic Xerorthent	7.0	1.20	43.0	1.8	22	54	24
Sharpsburg silty clay loam	Typic fine, montmorillonitic, mesic Argiudoll	6.5	1.10	89.2	0.9	3	63	34

† Mean of three replicates.

N₂O ratio could be used to estimate denitrification in the field solely from N₂O flux measurements.

MATERIALS AND METHODS

The soils used in this study, characteristics of which are given in Table 1, were collected from the A horizon of benchmark sites, three in Nebraska and one in California. Soil pH was maintained near neutral so as not to influence the gaseous N products being emitted from the soil. Biological activity was reestablished in stored soils by growing oats (*Avena sativa* L.) in large pots of each soil for 6 wk in the greenhouse. At the end of this period, the plants were removed and the soil sieved to pass 2 mm before air drying to a water content of 40% WFPS. Water-filled pore space is synonymous with relative saturation and was calculated as WFPS = [(gravimetric water content × soil bulk density)/total soil porosity], where soil porosity = [1 - soil bulk density/2.65] and 2.65 equals the assumed particle density of soil (Mg m⁻³).

Sixty grams of each soil (oven-dry basis) were weighed into 100-mL plastic vials and hand compacted to predetermined bulk densities (Table 1). Experimental treatments of C (0, 0.5, and 1.0 mg glucose-C g⁻¹ soil) and N (0, 139, and 277 µg KNO₃-N g⁻¹ soil) were added in solution to each container to attain WFPS of 60, 75, and 90%. The C and N application rates were equivalent to 0, 180, and 360 kg C ha⁻¹ and 0, 50, and 100 kg N ha⁻¹, respectively, based on soil depths of 3.0, 3.2, 3.3, and 3.6 cm for the Hord, Valentine, Yolo, and Sharpsburg soils, respectively, and a container diameter of 4.7 cm. Each treatment was replicated three times in a randomized complete block design. Discussion of treatments and results will be based on grams or kilograms per hectare to facilitate comparison with conditions in the field.

Each container and soil was placed in a 2-L glass jar and sealed with a screw-cap lid in which a septum had been fitted for gas sampling. In one-half of the jars, 100 mL L⁻¹ of the headspace was replaced by C₂H₂, while the other one-half contained no C₂H₂. The jars were incubated at 25 °C for 5 d. Gas samples of the headspace of all jars were taken daily for N₂O and CO₂ analysis using evacuated vials, after which the jars were flushed with atmospheric air and fresh C₂H₂ added to one-half of the jars before all jars were resealed and returned to the incubator. Control samples were included to correct for small amounts of N₂O contained in jars without soils. After the last gas sampling, the plastic containers were capped, placed in a mechanical shaker, and the soil core disrupted by shaking for 60 min. Gas samples were again taken to measure entrapped N₂O and CO₂ in the soil (Aulakh and Doran, 1990). Nitrous oxide was measured on a 1-mL sample using a gas chromatograph fitted with a 3.2 mm by 1.8 m stainless steel

column (packed with 50–80 mesh [0.18–0.28 mm diam.] Porapak Q) and a ⁶³Ni electron capture detector. Operating conditions were: column temperature, 50 °C; electron capture detector temperature, 350 °C; 5% CH₄/95% Ar carrier gas at a flow rate of 60 mL min⁻¹. Carbon dioxide was measured on a 1-mL sampling using a gas chromatograph with a thermal conductivity detector maintained at 110 °C. A 6.35 mm by 1.8 m Teflon column (packed with 50–80 mesh Porapak Q), at an oven temperature of 75 °C with He as carrier gas at a flow rate of 60 mL min⁻¹, was used to separate CO₂. Water vapor was removed from all samples with a precolumn of 8-mesh (2.5 mm diam.) CaCl₂. Results were corrected for the solubility of N₂O and CO₂ in soil water using the procedure of Moraghan and Buresh (1977). Mineral N was extracted from a 10-g soil sample from each container using 1 M KCl, and

Table 2. Denitrification rate of four soils during 5 d incubation at 25 °C as affected by soluble N and C, and soil water-filled pore space (WFPS).

Treatments			Average denitrification rate			
N	C	WFPS	Valentine sand	Hord silt loam	Yolo silt loam	Sharpsburg silty clay loam
kg ha ⁻¹	%		g N ha ⁻¹ d ⁻¹			
0	0	60	10 (2)†	13 (4)	10 (2)	7 (3)
0	0	75	44 (8)	18 (4)	1 (0.2)	1 (0.3)
0	0	90	76 (12)	203 (40)	98 (25)	87 (34)
0	180	60	7 (4)	14 (10)	9 (7)	11 (6)
0	180	75	25 (19)	91 (79)	96 (66)	156 (116)
0	180	90	71 (7)	195 (37)	1396 (672)	3850 (1256)
0	360	60	9 (7)	28 (24)	17 (10)	5 (4)
0	360	75	51 (28)	188 (65)	104 (76)	1654 (819)
0	360	90	79 (13)	187 (35)	1697 (590)	4214 (1739)
50	0	60	9 (2)	6 (2)	3 (1)	2 (1)
50	0	75	124 (35)	16 (3)	2 (1)	7 (4)
50	0	90	237 (58)	360 (122)	6 (3)	172 (62)
50	180	60	2672 (1207)	87 (67)	34 (22)	33 (23)
50	180	75	4770 (1772)	3684 (1106)	110 (84)	760 (418)
50	180	90	5367 (1681)	6287 (2029)	9041 (3692)	12652 (3489)
50	360	60	1300 (452)	541 (308)	121 (68)	23 (16)
50	360	75	6510 (3259)	3717 (2116)	2210 (1009)	1650 (960)
50	360	90	4923 (1708)	8814 (3419)	9935 (3959)	12669 (4279)
100	0	60	5 (1)	2 (1)	3 (1)	2 (1)
100	0	75	87 (23)	36 (7)	2 (1)	1 (0.3)
100	0	90	210 (55)	303 (105)	27 (11)	317 (114)
100	180	60	1309 (745)	70 (55)	8 (6)	2 (1)
100	180	75	8919 (2213)	1281 (450)	134 (79)	34 (26)
100	180	90	11272 (3464)	14227 (3713)	6514 (1597)	10565 (2509)
100	360	60	2606 (796)	230 (136)	93 (52)	13 (8)
100	360	75	13230 (3503)	10999 (5747)	773 (411)	268 (151)
100	360	90	9383 (2887)	26806 (7886)	17479 (5183)	28065 (9679)

† Standard error of mean in parentheses.

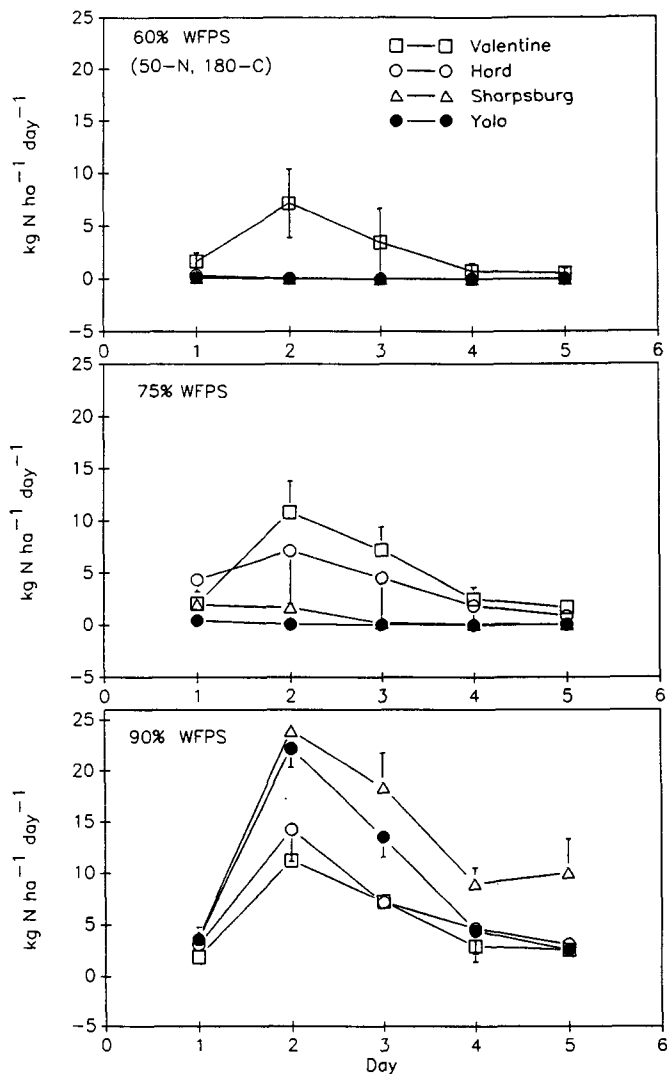


Fig. 1. Denitrification rate of four soils at 60, 75, and 90% water-filled pore space (WFPS) after the application of 50 kg N ha⁻¹ and 180 kg C ha⁻¹. Vertical bars show standard errors.

NO₃-N and NH₄-N determined colorimetrically (Keeney and Nelson, 1982).

The production of N₂O by denitrification was estimated by the amount of N₂O evolved from soil cores without C₂H₂, whereas total denitrification (N₂ + N₂O) was estimated from the amount of N₂O produced from C₂H₂-treated soil cores. The N₂/N₂O ratios were therefore calculated using N₂ amounts estimated by the difference in N₂O produced between C₂H₂-treated and untreated cores (Ryden et al., 1979).

RESULTS

Total denitrification in all four soils was influenced by the addition of glucose-C and NO₃, with the response varying among treatment combinations and soil WFPS. At 60% WFPS, <29 g N ha⁻¹ d⁻¹ was lost via denitrification in (i) the absence of both added N and C, (ii) the absence of N and the presence of C, and (iii) the presence of N and the absence of C. At 75 and 90% WFPS, total denitrification increased in all soils when

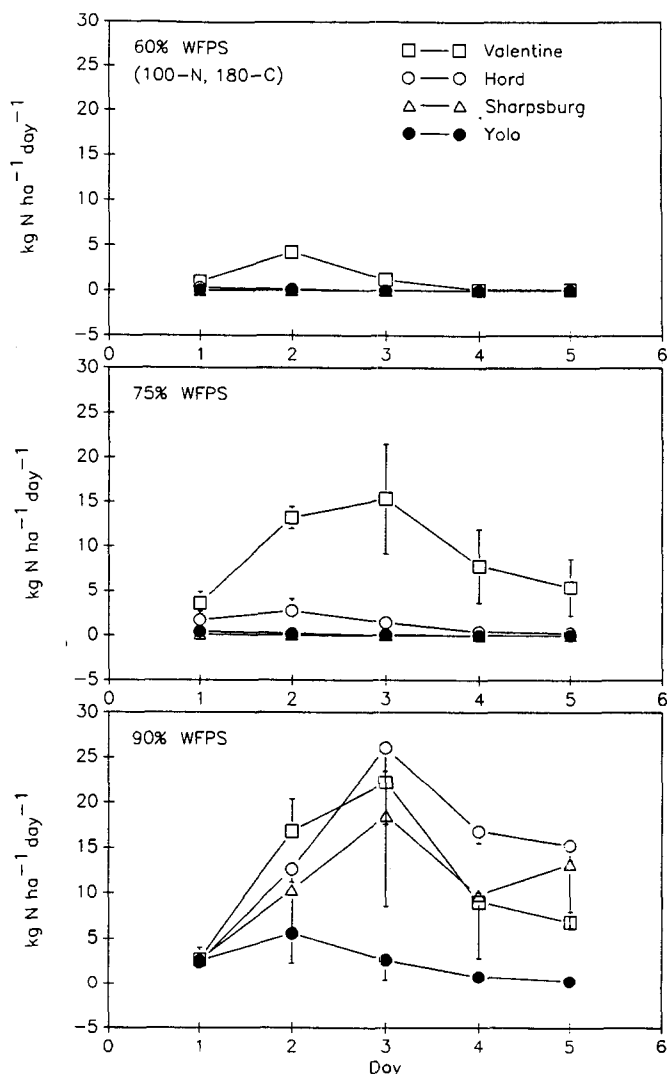


Fig. 2. Denitrification rate of four soils at 60, 75, and 90% water-filled pore space (WFPS) after the application of 100 kg N ha⁻¹ and 180 kg C ha⁻¹. Vertical bars show standard errors.

compared with that at 60% WFPS (Table 2). The maximum rates of production were observed in the Sharpsburg and Yolo soils, where initial soil NO₃ concentrations were high, but only after the addition of C (glucose) to the soils (Tables 1 and 2). The addition of N, in combination with C amendment, increased denitrification in all soils, but soil texture significantly influenced total N loss at different WFPS values (Fig. 1–3). For the Valentine sand, WFPS had very little effect on total denitrification and was highest of all soils at 60 and 75% WFPS for 50 kg N ha⁻¹ and 180 kg C ha⁻¹. At 90% WFPS, denitrification was greatest in the finer textured Sharpsburg and Yolo soils (Fig. 1). However, when N concentration was increased to 100 kg N ha⁻¹ at the same C concentration (Fig. 2), denitrification rate at 90% WFPS decreased in the Sharpsburg and Yolo soils (the latter significantly) and increased in the coarser textured Hord and Valentine soils. At 100 kg N ha⁻¹ and 360 kg C ha⁻¹, the additional C resulted in greater total N loss via denitrification from the Hord soil at 75 and 90% WFPS

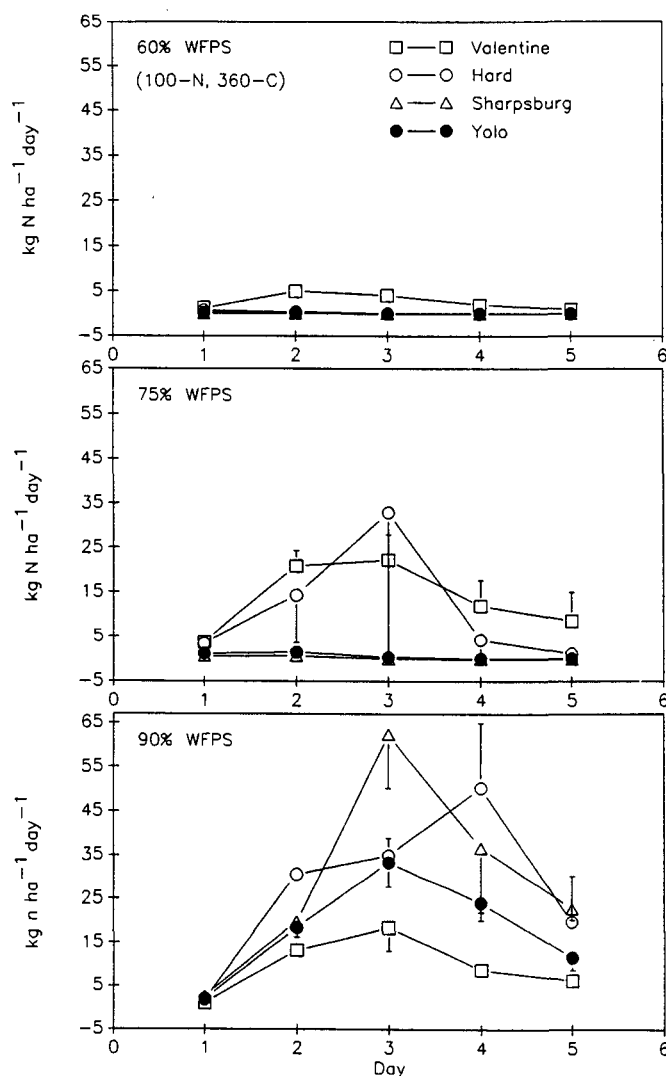


Fig. 3. Denitrification rate of four soils at 60, 74, and 90% water-filled pore space (WFPS) after the application of 100 kg N ha⁻¹ and 360 kg C ha⁻¹. Vertical bars show standard errors.

and the Sharpsburg and Yolo soils at 90% WFPS (Fig 3). For most treatments, uniform denitrification rates occurred after Days 3 and 4.

The percentage of added NO₃ lost via denitrification generally increased with increasing WFPS and added C. At 60% WFPS, the Valentine sand amended with N and C lost between 7 and 26% of the added N, while at 75% WFPS this had increased to 48 to 66% (Table 3). The Hord soil also exhibited a high N loss at 75% WFPS, but the greatest loss of N from this soil occurred at 90% WFPS. For the finer textured Sharpsburg and Yolo soils, large N losses generally only occurred at 90% WFPS. The percentage of N gases that remained entrapped in the soil solution at the end of the incubation varied between soils, with values ranging from 0 to 46% at 90% WFPS. At 60 and 75% WFPS, entrapped gas was found only in the Valentine soil, with maximum values of 0.4 and 25%, respectively.

As expected, microbial respiration rates increased significantly with the addition of C at all WFPS regardless

Table 3. Nitrate lost via denitrification from four soils during a 5-d incubation at 25 °C.

Treatments			NO ₃ -N lost by denitrification			
N	C	WFPS†	Valentine sand	Hord silt loam	Yolo silt loam	Sharpsburg silty clay loam
kg ha ⁻¹			%			
0	0	60	4.5 (2.7)‡	3.0 (.7)	0.3 (.2)	0.1 (.06)
0	0	75	20.8 (11.8)	4.1 (2.3)	0 (-)	0 (-)
0	0	90	46.1 (12.7)	61.6 (7.7)	3.1 (2.6)	2.4 (1.1)
0	180	60	3.2 (1.8)	3.2 (0.9)	0.3 (0.1)	0.1 (0.7)
0	180	75	11.5 (3.2)	20.7 (4.1)	2.8 (1.7)	2.2 (.9)
0	180	90	36.9 (17.2)	51.0 (18.7)	41.0 (8.0)	56.4 (6.4)
0	360	60	4.1 (2.7)	6.3 (3.6)	0.5 (.1)	0.1 (.01)
0	360	75	23.1 (10.5)	42.8 (16.4)	3.0 (.6)	23.1 (6.6)
0	360	90	40.6 (14.2)	47.7 (8.2)	43.2 (6.7)	60.7 (5.6)
50	0	60	0.1 (.06)	0.1 (.04)	0 (-)	0 (-)
50	0	75	1.6 (0.8)	0.2 (.08)	0 (-)	0 (-)
50	0	90	2.9 (0.7)	5.6 (1.7)	0 (-)	1.1 (0.7)
50	180	60	21.9 (17.8)	0.8 (0.3)	0.3 (.1)	0.2 (0.1)
50	180	75	49.3 (15.0)	24.8 (11.1)	0.8 (.2)	4.3 (4.1)
50	180	90	61.1 (2.7)	75.2 (9.7)	69.7 (4.4)	79.5 (0.2)
50	360	60	12.7 (3.8)	5.2 (2.8)	0.9 (.2)	0.1 (.05)
50	360	75	63.9 (4.9)	35.7 (20.5)	16.8 (7.3)	9.3 (4.2)
50	360	90	60.7 (3.1)	85.0 (14.6)	77.9 (18.3)	69.4 (12.1)
100	0	60	0 (-)	0 (-)	0 (-)	0 (-)
100	0	75	0.6 (.4)	0.2 (0.1)	0 (-)	0 (-)
100	0	90	1.9 (.4)	1.5 (0.01)	0.1 (.08)	2.1 (0.6)
100	180	60	6.5 (1.9)	0.3 (0.1)	0 (-)	0 (-)
100	180	75	48.2 (13.2)	6.3 (3.1)	0.6 (.1)	0.1 (.05)
100	180	90	71.3 (4.6)	90.1 (14.8)	9.8 (6.2)	42.0 (27.0)
100	360	60	12.9 (4.7)	1.1 (0.2)	0.4 (.2)	0.1 (.01)
100	360	75	67.1 (14.1)	53.8 (46.4)	2.7 (.2)	1.0 (0.4)
100	360	90	60.3 (8.6)	133 (7.8)	88.0 (3.5)	107 (15.7)

† WFPS = water-filled pore space.

‡ Standard error of mean in parentheses.

of N concentration (data not shown). There was an exponential decrease in respiration rate during the 5-d incubation period at 60 and 75% WFPS for all soils and all treatment combinations (all data not shown) while, at 90% WFPS, C addition resulted in increased respiration for 4 d, although values were much lower than in the better aerated soil (Fig. 4).

The N₂/N₂O ratios obtained from headspace measurements of N₂O production as a consequence of the various treatments are given in Tables 4 and 5. The largest ratio of 549 was found for the 50 kg N ha⁻¹ and 360 kg C ha⁻¹ treatment on the Valentine soil, although many other treatments (mostly in the Valentine and Sharpsburg soils) gave ratios of >100 (<1% in direct N₂O evolution). In all but two of these cases, however, high ratios occurred in soil to which 360 kg C ha⁻¹ had been added. With the exception of the Yolo soil at 90% WFPS, all soils without added C exhibited low N₂/N₂O ratios than those with C regardless of water content. The N₂/N₂O ratio also changed with time after the application of the treatments, being smaller initially and increasing with time of incubation. Increasing WFPS also resulted in a greater scatter of N₂/N₂O ratios, with the higher C concentrations producing the largest ratios. However, the average ratios for the 100 kg N ha⁻¹ and 360 kg C ha⁻¹ treatment were lower than those found for the other N and C treatment combinations and ranged from 0.1 at 60% WFPS to 51.6 at 90% WFPS.

DISCUSSION

The onset of denitrification in these soils, after the application of the various treatment combinations, was

Table 4. Ratios of N_2/N_2O produced from denitrification during 5 d as calculated from measurement of N_2O in the headspace of C_2H_2 -amended and nonamended Valentine and Hord soils.

Treatments			N_2/N_2O Ratios†									
			Valentine sand					Hord silt loam				
N	C	WFPS‡	1 d	2 d	3 d	4 d	5 d	1 d	2 d	3 d	4 d	5 d
— kg ha ⁻¹ —		%										
0	0	60	—§	0.2	—	1.2	1.2	0.4	1.1	—	5.9	0.7
0	0	75	1.4	4.1	4.3	3.5	0.3	6.0	2.5	3.6	3.0	1.1
0	0	90	3.9	2.3	2.8	2.4	2.1	13.1	5.8	2.0	1.5	1.4
0	180	60	1.3	—	2.1	1.2	—	2.0	0.1	—	0.8	—
0	180	75	16.0	—	0.7	—	—	14.8	6.6	0.6	—	—
0	180	90	58.0	251.3	9.4	13.3	6.0	39.3	55.9	45.8	11.2	22.3
0	360	60	17.4	—	—	—	—	27.9	0.9	—	0.2	—
0	360	75	19.4	36.1	86.3	4.5	—	60.2	146.2	17.4	11.7	4.1
0	360	90	30.4	69.3	29.5	15.1	4.9	9.7	15.1	11.9	16.9	12.0
50	0	60	—	—	—	0.2	0.8	—	0.5	19.9	2.6	1.3
50	0	75	0.1	1.3	2.7	5.2	5.1	0.2	—	—	—	—
50	0	90	0.9	3.1	4.2	4.5	4.7	—	1.3	2.1	2.1	3.5
50	180	60	3.5	3.8	9.2	9.9	17.4	0.3	0.3	—	0.1	1.2
50	180	75	2.2	2.8	6.5	14.1	17.8	0.8	1.3	4.1	5.1	6.7
50	180	90	1.9	2.1	3.7	5.5	6.5	2.4	2.7	4.1	6.1	14.3
50	360	60	2.1	0.3	0.8	3.6	4.7	2.4	2.9	1.0	0.9	1.0
50	360	75	3.5	4.0	2.9	1.9	5.0	1.4	1.7	3.7	4.0	2.9
50	360	90	22.3	167.7	549.0	275.1	245.1	2.3	36.4	156.8	93.8	56.0
100	0	60	—	0.5	5.0	0.2	0.4	—	—	—	—	—
100	0	75	—	—	0.1	1.1	1.4	0.8	3.5	8.7	1.4	0.7
100	0	90	9.8	2.2	2.0	2.1	1.9	—	0.2	0.8	0.7	1.3
100	180	60	1.4	2.3	2.8	2.1	4.2	0.1	—	—	0.2	0.5
100	180	75	1.5	1.0	2.1	2.6	8.6	—	—	—	—	0.3
100	180	90	2.4	2.3	0.5	1.2	1.7	1.1	0.9	1.6	1.1	1.7
100	360	60	3.6	0.4	—	1.0	0.6	0.6	1.6	1.7	1.0	0.7
100	360	75	3.5	1.7	1.0	1.2	0.9	0.4	2.1	28.7	18.4	12.2
100	360	90	1.0	2.0	1.7	1.3	2.5	0.3	2.0	6.2	29.8	51.6

† WFPS = water-filled pore space.

‡ To calculate the percentage of ($N_2O + N_2$) emitted as N_2O from the soil, use the factor $[1/(1 + N_2/N_2O)] \times 100$.§ Negative values for N_2/N_2O ratio due to greater production of N_2O from non- C_2H_2 - than C_2H_2 -treated soil cores.**Table 5. Ratios of N_2/N_2O produced from denitrification during 5 d as calculated from measurement of N_2O in the headspace of C_2H_2 -amended and nonamended Yolo and Sharpsburg soils.**

Treatments			N_2/N_2O Ratio†									
			Yolo silt loam					Sharpsburg silty clay loam				
N	C	WFPS‡	1 d	2 d	3 d	4 d	5 d	1 d	2 d	3 d	4 d	5 d
— kg ha ⁻¹ —		%										
0	0	60	—§	1.4	3.4	5.2	0.6	—	—	6.7	3.7	0.5
0	0	75	0.4	—	—	—	—	—	1.9	—	—	0.6
0	0	90	—	48.1	49.6	36.9	48.8	1.2	—	1.2	1.0	—
0	180	60	2.6	—	—	—	—	20.9	2.0	—	5.3	7.1
0	180	75	67.3	—	0.7	4.1	3.4	434.0	36.4	2.7	0.1	0.8
0	180	90	6.2	8.5	4.2	7.3	6.9	3.2	5.8	6.2	13.8	—
0	360	60	10.6	5.5	—	11.3	0.2	0.5	—	—	—	—
0	360	75	32.9	57.6	4.0	—	—	114.6	—	116.6	7.1	17.3
0	360	90	33.7	82.0	32.3	48.0	35.9	3.8	3.7	29.0	18.4	19.3
50	0	60	—	1.4	—	0.1	—	1.5	—	1.1	—	2.1
50	0	75	1.1	0.2	1.5	2.9	—	—	—	12.5	3.6	2.7
50	0	90	—	—	—	0.4	2.1	0.3	3.5	2.1	0.8	—
50	180	60	2.6	11.2	0.5	—	—	13.5	11.6	—	—	—
50	180	75	0.8	—	0.1	—	—	19.0	—	—	62.7	3.1
50	180	90	1.2	1.7	5.7	9.0	16.7	0.7	0.9	0.6	1.0	2.7
50	360	60	2.0	2.7	0.8	—	—	0.6	0.9	—	—	0.3
50	360	75	3.2	3.4	17.0	17.4	13.8	1.8	1.0	—	—	—
50	360	90	0.4	0.6	3.5	8.7	17.0	1.1	1.8	11.8	23.9	31.0
100	0	60	0.3	0.6	—	—	—	—	—	2.3	1.7	—
100	0	75	—	—	16.7	0.4	—	—	—	—	—	1.7
100	0	90	—	4.2	1.5	10.4	18.7	—	1.9	6.3	9.3	10.7
100	180	60	—	—	0.2	—	—	—	—	—	—	—
100	180	75	0.7	1.4	2.0	0.8	1.4	0.7	3.2	—	—	—
100	180	90	—	0.9	2.3	9.3	23.1	0.1	—	—	0.1	—
100	360	60	3.5	2.8	4.8	0.5	0.2	—	1.6	—	—	0.2
100	360	75	2.8	1.4	3.2	2.9	0.5	0.2	1.3	—	—	—
100	360	90	0.3	—	0.1	3.1	9.6	0.1	0.6	0.1	1.8	5.6

† WFPS = water-filled pore space.

‡ To calculate the percentage of ($N_2O + N_2$) emitted as N_2O from the soil, use the factor $[1/(1 + N_2/N_2O)] \times 100$.§ Negative values for N_2/N_2O ratio due to greater production of N_2O from non- C_2H_2 - than C_2H_2 -treated soil cores.

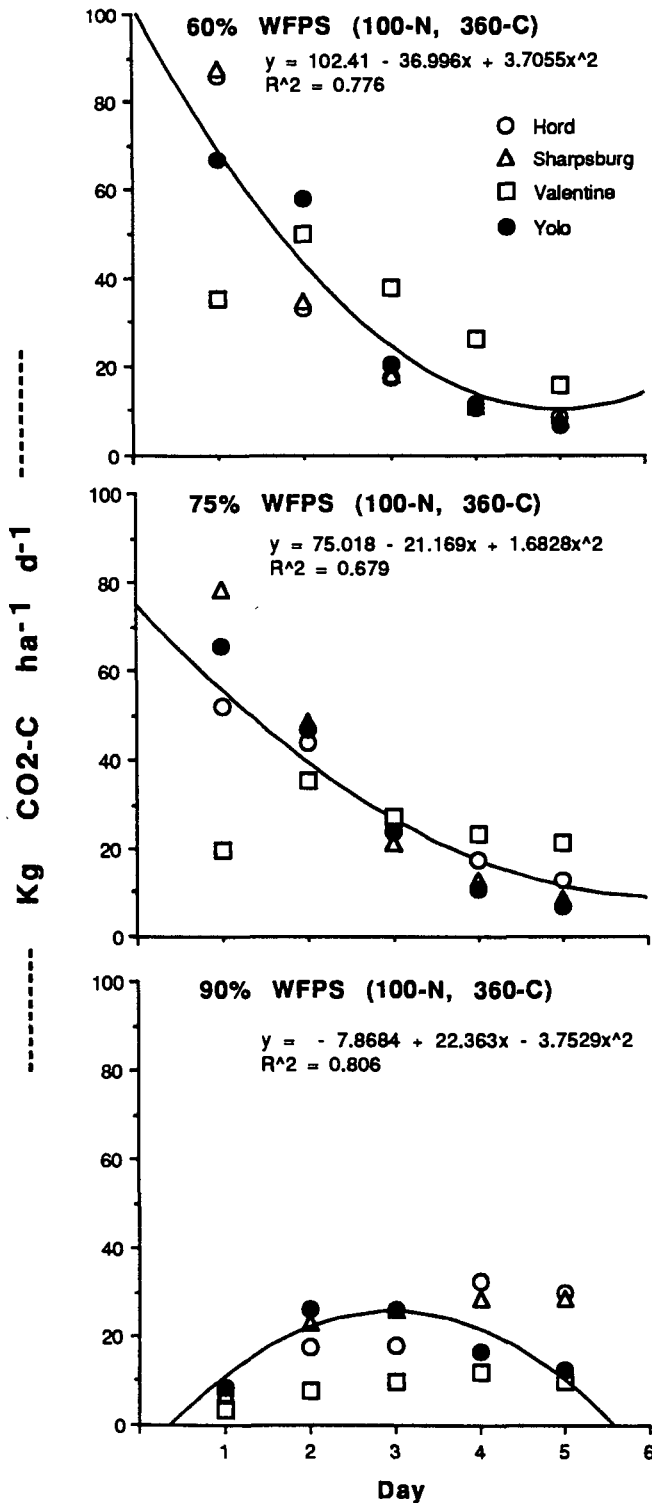


Fig. 4. Respiration rate of four soils at 60, 75, and 90% water-filled pore space (WFPS) after the application of 100 kg N ha⁻¹ and 360 kg C ha⁻¹.

apparently influenced by the physical structure of the soils. For the Valentine sand at 60 and 75% WFPS, and the Hord wild loam at 75% WFPS, the greater total gaseous N loss may have been caused by excess water in-

hibiting O₂ diffusion to the soil microorganisms, thus creating an environment favorable for denitrification (Nommik, 1956). As both fineness of soil texture and WFPS increased, total denitrification also increased, as evidenced by data for the Sharpsburg and Yolo soils. Groffman and Tiedje (1989) suggested that smaller average pore size in finer textured soils may lead to greater soil water retention and greater opportunity to create anaerobiosis. They found no denitrification in a poorly drained sandy soil.

The addition of glucose-C to these soils greatly increased denitrification of NO₃-N, while the addition of N alone had little effect. This supports the well-known fact that microorganisms require a readily decomposable substrate before reduction of added NO₃ can occur (Burford and Bremner, 1975). Without additional C, NO₃ accumulation occurs. High NO₃ can result in the inhibition of N₂O reductase activity (Firestone et al., 1979), which reduces gaseous N production (Lalisse-Grundmann et al., 1988) and also the conversion of N₂O to N₂ (Blackmer and Bremner, 1978).

Changes in N₂ and N₂O evolution rates are often associated with changes in NO₃ concentration in soils. As the percentage of NO₃ lost via denitrification increases, the N₂/N₂O ratio also increases and N₂ becomes the principal gas evolved. Letey et al. (1980) found no such relationship but offered no explanation as to why their results differed from those found in other studies (Nommik, 1956; Rolston et al., 1978). The quantity of denitrification gases entrapped in the soil at 90% WFPS in our study is similar to that found by Aulakh and Doran (1990) but higher than that found by Holt et al. (1988). However, entrapment of N gases also occurred at 75% WFPS in the Valentine sand, and this result had not been previously reported.

An exponential decrease in soil respiration with time at 60 and 75% WFPS in our study, regardless of the N and C treatments applied, suggests a rapid initial fluid of respiration that subsequently increased denitrification due to increased O₂ uptake. The slight increase in CO₂ production with time for the 90% WFPS and 360 kg C ha⁻¹ treatment may have been caused by the proliferation of anaerobic bacteria, the restriction imposed on aerobic microbial respiration by high WFPS being compensated for by an increased level of available C to fermenters.

The largest N₂/N₂O ratios found for N gases emanating from these soils occurred at the higher WFPS, particularly with the highest glucose-C treatment. This finding is consistent with other research, which indicated that, with increasing anoxic conditions, the percentage of N₂O in denitrification products decreases (Rolston et al., 1978) until N₂ is the major gas evolved. Blackmer and Bremner (1976) also suggested that the addition of glucose-C promotes the growth of soil microorganisms and increases uptake of N₂O in soils. However, in our study, smaller ratios were obtained with increasing NO₃ concentrations, which suggests that higher NO₃ concentrations may inhibit the conversion of N₂O to N₂ (Blackmer and Bremner, 1976). The latter effect was particularly noticeable at 90% WFPS, but seemed to be moderated by the presence of the higher glucose concentration as larger ratios appeared after 2 to 3 d. The N₂/N₂O ratio also changed with time after application of treatments, being smaller initially, and then larger after a few days. This was not

always the case, however, as N_2 was the principal gas initially evolved on more than one occasion.

The N_2/N_2O ratios found for these soils are much higher than those previously reported Rolston et al., 1976; Ryden et al., 1979), and indicate that N_2 is the major gas being emitted from these soils. However, the ratios do show that, in some treatments, more than 90% ($N_2/N_2O < 0.1$) of denitrification products may occur as N_2O .

The relatively large numbers of negative N_2/N_2O ratios in our study indicate the limitations of using C_2H_2 inhibition to determine the relative amounts of N_2O and N_2 produced during denitrification. While the total N_2O produced in the presence of C_2H_2 may be a good indicator of total denitrification ($N_2 + N_2O$), the production of N_2O in the absence of C_2H_2 may not be solely a product of denitrification but may include nitrification and the other aerobic processes as well. Thus, it is not surprising that, in some cases, the production of N_2O without C_2H_2 exceeded that produced with C_2H_2 ($N_2 + N_2O$), resulting in negative calculated values for N_2 production and a resultant negative N_2/N_2O ratio.

We conclude that denitrification in the benchmark soils studied is largely regulated by soil water status and the availability of the C source. We also found that an average N_2/N_2O ratio cannot be used to convert N_2O measurements in the field to estimates of total denitrification because of the variability in the environmental factors that control the denitrifying process. The results from the glucose-C and N amendments do indicate, however, that the proportions of N_2 and N_2O entering the atmosphere from soil can be controlled by changes in management practices. Application of N fertilizer more frequently and at lower rates will reduce the NO_3 concentrations in soil and increase the conversion rate of N_2O to N_2 . The addition or presence of an easily decomposable substrate in soil when soil water contents are high will increase anoxic conditions and again increase the conversion rate of N_2O to N_2 . Both of these practices may result in less N_2O entering the atmosphere.

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